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Anticholinesterases

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Alice in Wonderland
[illegible]
The Commando'

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Any account of the anticholinesterase drugs must begin by taking into consideration the properties and the distribution of the cholinesterase enzymes as well as of the drugs which inhibit them. This immediately leads us into ground so complicated that one is in danger of losing the original and rather straightforward background to these agents. It is therefore worth while to outline this basic story and to consider the later developments as elaborations or divergencies from it.

The preliminary rumblings are heard in Fraser's study of the toxicity of the esere bean in 1860–70, and of the antagonism of atropine to it; we now know that the Calabar natives in their choice of an ordeal poison had anticipated modern chemical warfare by many years. Then comes the observation by Dale in 1914 of the brevity of the action of acetylcholine, prompting his suggestion that an enzyme exists in the body able to destroy acetylcholine. Loewi and Navratil (1926) showed that 'Vagusstoff' (later to be identified as acetylcholine) could be rapidly destroyed, and in 1930 Engelhardt and Loewi found that eserine would prevent this. About this time, the first chemical analogues of eserine were being made by Stedman and Aeschliman and his colleagues, amongst them neostigmine. It is interesting that they were developed primarily as miotics and were only later recognized as anticholinesterases. From 1934 onwards came the fundamental experiments of chemical transmission. These elucidated how eserine produces its typical effects, by preserving acetylcholine released at nerve-endings, so favouring transmission, unless the accumulation of transmitter went to excess and began itself to cause synaptic block.

Given these facts of cholinergic transmission and appreciating the importance of an enzyme for destroying acetylcholine once released (to allow independent repeated shocks, and to prevent block by accumulation) then one might expect to describe the situation in the following simple terms:

(1) there would be a single enzyme whose action is specific to acetylcholine and which acts on acetylcholine with great rapidity and in high dilution;

(2) this enzyme would be localized at the sites of acetylcholine action only:

(3) the enzyme would be specifically antagonized by a number of inhibitors which resemble the transmitter chemically;

(4) the effect of these inhibitors of the enzyme would all be due to the accumulation of unhydrolysed acetylcholine and the intensity of their effects would be in proportion to the degree of enzyme inhibition.

As a framework for the discussion we shall take each of these simple statements in turn, and see how far they have come to need expansion or qualification.

The Enzymes Destroying Acetylcholine

It was quite early recognized that more than one enzyme in the body could destroy acetylcholine. Since these observations were made, a considerable literature has grown up, a good deal of which is concerned with what the enzymes should be called (for reviews see Augustinsson (1942) and Whittaker (1951)). This has tended to make the subject rather confusing, but it can be simplified somewhat if one relates each proposed nomenclature to the particular point of interest of the investigator concerned. First was Mendel and Rudney, who studied two crude enzymes both of which could at first split aliphatic esters as well as choline esters. When they purified these preparations, however, they found that one ceased to hydrolyse the aliphatic esters, while the other continued to do so. On the basis of this and other evidence they concluded that the first enzyme was really and truly concerned with hydrolysing acetylcholine so that they named it 'true' cholinesterase, while the second was only apparently concerned with this and hence was named 'pseudo'-cholinesterase. They further showed that one could measure the true cholinesterase in a tissue or extract by the rate of hydrolysis of acetyl-β-methyl-choline (a substance chemically very like acetylcholine), and the pseudo-cholinesterase by the rate of hydrolysis of benzoylcholine (a rather different compound). This christening was soon challenged, partly because words like 'true' and 'pseudo' had an air of moral judgement, and partly because it was found that some cholinesterases existed whose properties diverged from those laid down. But these names reflect a useful physiological approach, and though they may be criticized, their use is convenient, nearly self-explanatory and not fundamentally misleading, since the conception implied has been generally accepted, even if the names have not.

Other workers found that the 'true' cholinesterases could hydrolyse a number of substances possessing acetyl groups but not choline groups, so that they thought that a better name for the true enzyme would be 'acetylase'. A third group of workers analysed the kinetics and specificity of the enzymes, stressing (1) the high rate of reaction of the true enzyme with acetylcholine, (2) the fact that it seemed designed to work most efficiently in low dilution, and (3) the fact that it seemed to be fairly particular about the resemblance of its substrates to acetylcholine. They painted a picture whereby true cholinesterase seemed to bear a highly specific relationship to acetylcholine while the pseudo-enzyme was not *specifically* concerned with it; hence the names 'specific' and 'non-specific' cholinesterase. A fourth approach arose from experiments varying the aliphatic chain in acetylcholine. This revealed that 'true' split acetyl esters more rapidly than any other ester, while the 'pseudo' split butyric esters much better. Hence the names 'aceto-cholinesterases' and 'butyro-cholinesterases'. A fifth approach is to abandon attempts to give an explanatory name and simply refer to them as 'Group I' and 'Group II' enzymes, or 'α' and 'β' enzymes, names which could obviously be retained whatever properties were revealed about the enzymes. Another approach of the same sort was simply to state where the enzyme came from or at least where the prototype enzyme came from. Thus the 'true' enzyme was called 'E-cholinesterase' for erythrocyte cholinesterase and the 'pseudo' was called 'S-cholinesterase' for serum cholinesterase.

One is reminded of the White Knight in *Through the Looking Glass* (Carroll, 1878), where he explains to Alice that the song he is about to sing has a name *called* 'Haddocks Eyes', although its name *really is* 'The Aged, Aged Man', but the *song* is called 'Ways and Means' and the song *really is* 'Sitting on a Gate'. The names given to the enzymes splitting cholinesterase could well be regarded in the same light.

Significant points from all this work could be summarized as follows. First, there is one enzyme (or possibly a group of very closely related enzymes) which (*a*) acts only on esters closely related to acetylcholine such as acetyl-β-methyl-choline; (*b*) acts very rapidly on acetylcholine; and (*c*) is favoured over an important range of concentration by dilution of acetylcholine (so that it actually comes to work less effectively when the concentration of acetylcholine rises). Second, there is also another and rather more puzzling family of pseudo-cholinesterases which split acetylcholine less rapidly, do it more efficiently the more concentrated the acetylcholine (thus working relatively inefficiently at low dilutions), and typically hydrolyse benzylcholine and butyric esters, although different members of the family differ somewhat in their favourite food. These facts do not matter much clinically, but they are fundamental in many investigations when one is trying to find out on what enzyme an inhibitor is acting, when it produces its characteristic effects.

The Distribution of Cholinesterases

Until recently acetylcholine's function in the body was viewed as being concerned solely with the transmission of activity across the various synapses, where, in fact, cholinesterase can be found. The most beautiful example is that of cholinesterase at the motor endplate, where it has been shown by a number of histochemical procedures (Koelle, 1951; Denz, 1953). It can also be identified in the cholinergic electric organ of *Electrophorus*, in cholinergic nerves, in autonomic ganglia (chiefly but not only localized in the preganglionic nerve terminals), in the adrenal medulla, in the central nervous system (particularly the basal nuclei) as well as in ganglia in the intestine. These sites are reasonable; within nervous tissue, particularly in the brain, its presence can be taken as a sign (along with choline-acetylase activity, and the presence of acetylcholine itself) of cholinergic activity by the nerves in which it is found. But it is less easy to understand why an enzyme conforming accurately to true cholinesterase should also exist in two nerve-free tissues, the placenta and the red cell, as well as in such unexpected materials as cobra venom (Koelle, 1951; Whittaker, 1951).

Pseudo-cholinesterase has a wider, indeed almost universal, distribution.

Our simple picture of an enzyme being localized only at the sites of acetylcholine released must therefore be modified; and we have instead to think of 'true' cholinesterase being localized at cholinergic synapses in the way expected, but also of true cholinesterase also occurring in other places, and of pseudo-cholinesterase being distributed very widely indeed (for reasons still not understood). A number of suggestions about the role of the latter can be made; if, as Professor Burn has suggested, acetylcholine is concerned in spontaneous rhythmic activity of tissues such as heart muscle, then pseudo-cholinesterase might have some important non-synaptic role there. Or secondly, it may be that pseudo-cholinesterase 'backs up' the true enzyme, particularly if (as Koelle (1953) points out) it can help the latter when high concentrations of acetylcholine occur. But it is much too early to discuss this in detail.

The Inhibitors of Cholinesterase

A useful way of summarizing these is to relate pharmacological action to their structural resemblance to acetylcholine (Fig. 1). It is now believed (largely from Nachmanssohn, Wilson and Bergmann's work on *Electrophorus* esterase) that the molecule of acetylcholine reacts with the enzyme at least at two sites: an anionic site, negatively charged, which associates with the

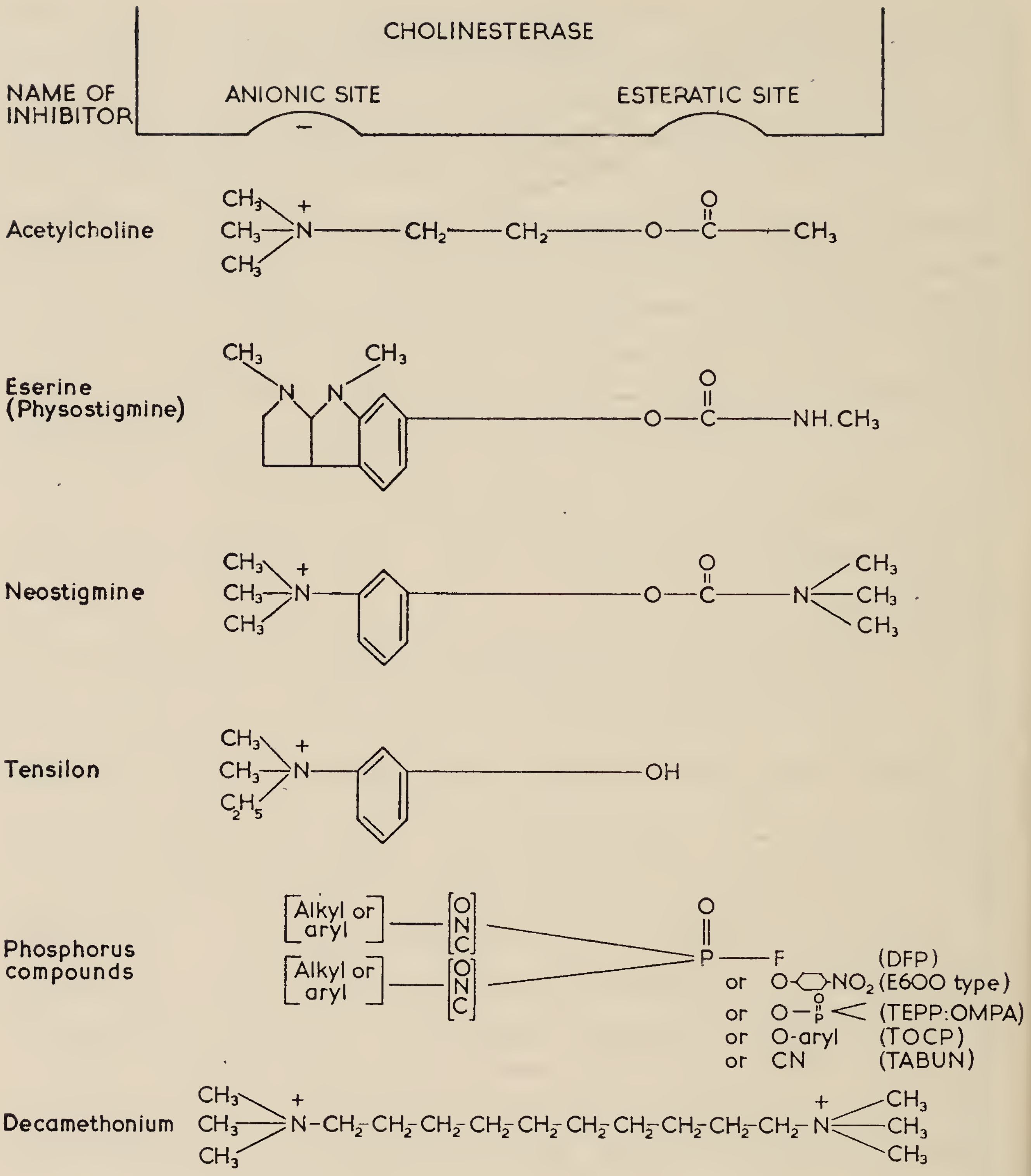


FIG. 1

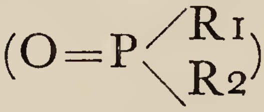
quaternary head, and an esteratic site, at which the split takes place, with an affinity for the ester arrangement of atoms.

If we consider *eserine* first, we see that it fits fairly well into both sites, while not having identity with acetylcholine at either position. It is therefore well-equipped to *compete* with acetylcholine for the receptor sites, and (incidentally) also to be itself slowly hydrolysed by the enzyme.

Neostigmine is in one structural respect closer to acetylcholine, by possessing a quaternary head. Here we must recall that this head seems to confer an ability to stimulate the endplate (seen outstandingly in decamethonium and succinylcholine) or sometimes to paralyse it. Therefore we are not surprised to find that it is a powerful antiesterase, but that it can also exert a decamethonium-like and, perhaps, with long-continued dosage, a curare-like action at the endplate. Notice also that it is quaternary; this means that it cannot cross the blood-brain barrier easily and so is relatively free of the central effects exerted by eserine on the one hand and fluorophosphates on the other.

Third is *Tensilon*; this looks definitely inferior so far as equipment for esteratic competition goes: and in fact it is not a highly active anticholinesterase. It has, as you would expect, some muscle stimulant action, and some curare action under big doses and the right conditions. But a new element appears here —that of sensitization of the endplate independent of antiesterase action: for instance, it favours decamethonium action quite strongly (Paton, 1951). As a consequence, its anticurare action is out of all proportion to its anticholinesterase action. Both Pelikan, Smith and Unna (1954) and Hobbiger (1952) maintain, however, that its antiesterase action accounts fully for its anticurare action: perhaps one had better suspend judgement on this particular point at present. Be that as it may, Tensilon differs in having even more of a direct muscular action han neostigmine, and in having a notably quicker and more ransient action (perhaps because it is a smaller molecule), but t resembles neostigmine in being free of central action.

An amazing product is the latest compound, 3113CT, which onsists of two neostigmine molecules linked by an aliphatic hain (Funke, Depierre and Krucker, 1952). It can inhibit og's red-cell cholinesterase at concentrations of 10^{-14} to 10^{-15} compared to eserine 10^{-9}), and is able to produce muscarinic igns of cholinesterase inhibition with a dose of a few microrams per kilo. It will be fascinating to see what develops from his.

Now all the compounds so far described are nitrogenous ases, competing reversibly with acetylcholine. The last category, developed partly for military purposes, partly as insecticides, a very wide one which is condensed enormously in the table, is of quite a different kind usually termed 'irreversible'. They are all compounds of organic phosphorus, and are believed to act by splitting off phosphonium ion ($O{=}P\genfrac{}{}{0pt}{}{\diagup R_1}{\diagdown R_2}$) which then phosphorylates the esteratic site. One can represent the situation by comparing cholinesterase to an attractive girl at a dance who usually breaks (hydrolyses) all hearts. Then one evening she finds a partner with a great mutual affinity (say eserine) who temporarily stops her multiple flirtations, by dancing with her all evening (his own heart being only broken a little bit); but when the dance is over, they separate and go home—the inhibition was reversible. With the phosphorus compounds it is quite different, and more dramatic, a suicide pact, in which both the partners are destroyed for ever. With DFP for instance, it seems that the enzyme has to be regenerated before activity returns, although with some of its relatives, such as TEPP and Paraoxon, the affair is reversible in its early stages.

This tendency to irreversibility is the first characteristic of the phosphorus compounds—bringing with it, of course, a much longer duration of action. But we can also predict (as is the case) that these drugs will be free from direct muscular actions—because they lack a quaternary group entirely. Further, since they lack the highly soluble nitrogenous group, they are liable to be fat soluble, sometimes actually volatile, and hence well absorbed into the lungs or from the skin, the gut, the eyes, and able to penetrate into and be taken up in the brain. They tend also to be 'fixed' in the tissues: for instance neostigmine injected into an arm exerts systemic effects, but DFP, in a comparable or larger dose, does not (Harvey *et al.*, 1947). There is one last important point: one might ask what happens when you combine the two types of anticholinesterase. If you give a phosphorus compound, then eserine or neostigmine subsequently simply adds on to it a little. But if you give eserine or neostigmine first, it seems to prevent the uptake of the irreversible inhibitor; and one gets the interesting situation that a small dose of reversible inhibitor will actually protect against several lethal doses of the other. Returning to our earlier metaphor, one can say that an esteratic flirtation will protect against a fatal passion!

The phosphorus inhibitors vary, of course, among themselves in several respects. *First*, their lipoid solubility; this is greatest for DFP, then paraoxon, then TEPP and eserine, then OMPA and neostigmine. This corresponds roughly to their liability to cause central effects (Burgen and Chipman, 1952). *Secondly*, their duration of action: some data on the duration of meiosis after application to the eye serve to illustrate this: the effects of DFP lasted 450 hours, TEPP 190, eserine 80, neostigmine 30. *Thirdly*, some of them proved to be much less active *in vitro* than *in vivo*. This is because such compounds are metabolized in the liver to a more active (although less stable) product; this has now been shown for Parathion (Aldridge and Davison, 1952), and OMPA (Dubois *et al.*, 1951; Gardiner and Kilby, 1952). It has even been shown that removing he liver can protect an animal against several lethal doses f OMPA (Cheng, 1951). The others, such as DFP, TEPP, nd the bases, have roughly proportionate actions under ll conditions. *Fourthly*, different inhibitors vary somewhat in heir proportionate antagonism to true and pseudo-esterases. FP for instance can inhibit particular pseudo-esterases in bout 200 times smaller concentration than that needed to inibit a true enzyme (v. Austin and Berry, 1953). This is often elpful in studying the enzymes, and it explains, or helps to xplain, why DFP sometimes is less active than expected; one an suppose that it is first taken up and fixed by the plasma nzyme, without having a chance to get at true enzyme localed at more strategic points. There is, unfortunately, no great gularity of behaviour in these proportionate antagonisms, and metimes the discrimination between pseudo and true is much ghter. Conversely, some compounds interfere with true olinesterase rather more readily, particularly the quaternary lts 284C51, and Nu 1250 (Austin and Berry, 1953); in fact camethonium has some action of this sort (Paton and Zaimis, 49). It may be possible, ultimately, to exploit these differces: but of course quaternary salts tend to have a good many other actions in addition to antiesterase effects, which have complicated this sort of progress. *Fifth*, it must be mentioned that the phosphorus compounds do not only inhibit cholinesterases but also some other enzymes, the so-called aliesterase, the proteolytic enzyme chymotrypsin, and the B-type esterase of mammalian sera (Mendel, Myers, *et al.*, 1953).

Our simple picture, therefore, of reversible specific inhibitors chemically related to acetylcholine can well be retained, provided we remember the two types of attachment to the enzyme, and allow for a combination of inhibitor with receptor which is at least so slowly reversible as to be, for practical purposes, permanent. There are, however, important complications: most inhibitors are pretty active against both enzymes, a few much more against one than the other; and cholinesterase is not the only type of enzyme inhibited.

There is one inhibitor of cholinesterase particularly active against pseudo-esterase, which comes in a category by itself, viz. triorthocresylphosphate (TOCP). This has been known for some years to produce a central nervous paralysis; it was later found to be an inhibitor of cholinesterase and later still found to be particularly active against pseudo-cholinesterase in the brain (Earl and Thompson, 1952). The interest in it arises from the fact that it produces central nervous system lesions of a kind also seen with two of the anticholinesterases, which will be discussed later.

Effects of Inhibition of Cholinesterase

It is fairly straightforward to interpret most of the effects seen in the terms of our original simple picture as being due to accumulation of acetylcholine, so that the results of inhibiting cholinesterases in the body provide a concise exercise in the anatomy of cholinergic nerves. The careful studies by Harvey and his colleagues on the effect of DFP on man, still provide one of the best descriptions of these effects. (Table 1.) The most prominent effects are the muscarinic ones. Of the nicotinic actions of acetylcholine only that on striated muscle reveals itself at all obviously. Remaining is a group of central nervous system actions, some of a quite remarkable kind.

There has been a good deal of discussion as to whether the

Table 1. Symptoms Following the Daily Intramuscular Administration of DFP
(From D. Crob, J. L. Lilienthal, A. M. Harvey and B. F. Jones (1947) *Bull. Johns Hopk. Hosp.* **81**, 217–44.)

Effector organ	Symptom	No. of subjects (Total: 50 normal and 10 myasthenic)
1. Muscarinic		
(*a*) Gastro-intestinal		41
	1. anorexia-nausea	29
	2. abdominal cramps	25
	3. vomiting	14
	4. diarrhoea	12
	5. cardiospasm	4
	6. nausea after smoking	4
(*b*) Sweat glands	increased sweating	10
(*c*) Lacrymal glands	increased lacrymation	3
(*d*) Salivary glands	increased salivation	2
(*e*) Pupils	slight miosis	2
(*f*) Ciliary body	difficulty of distant vision	2
(*g*) Lungs	respiratory difficulty, suggestive of bronchoconstriction	2
(*h*) Bladder	urinary frequency	2
(*i*) Heart	slight bradycardia with premature contractions	1
2. Nicotinic		14
Skeletal muscles	fasciculations (non-myasthenics only)	7
	increased strength (myasthenics only)	10
	decreased strength (non-myasthenics only)	4
	muscular cramps	1
3. Central nervous system		49
	excessive dreaming	33
	insomnia	29
	jitteriness, restlessness, increased tension, emotional lability, tremulousness	29
	nightmares, frequently with talking in sleep	17
	headache	8
	increased libido	6
	giddiness	6
	drowsiness	5
	paresthesias	3
	mental confusion	2
	visual hallucinations	1
	tremor	1
	pain in legs, sciatic distribution	1
4. Skin		
	urticaria (may have been sensitive to peanut oil)	2
5. No symptoms		6

phosphorus inhibitors exert a direct action, as well as causing acetylcholine to accumulate. Douglas and Paton (1954) went into this in some detail with TEPP so far as the neuromuscular junction is concerned, and failed to find any evidence for this; but some interesting points in the progression of TEPP poisoning emerged. Suppose you record the electric potential along a muscle fibre; normally it is the same all the way along. But if you give a drug such as decamethonium or acetylcholine, then the endplate regions become depolarized (i.e. negative to the rest of the fibre).

If TEPP is given, there develops a similar depolarization, but it is subject to an irregular waxing and waning depending on the amount of activity in the motor nerve. We know from the analysis of the effects of such depolarization that they will cause muscle fibres at first to contract, and later become inexcitable if it persists: so that you will get with such a fluctuation the sort of mixture of fasciculation and neuromuscular block which you see clinically. With a bigger dose a much larger, more prompt, and more persistent effect is seen. Repetitive stimulation of a given motor nerve produces a big increase in the depolarization: all these effects can be attributed to preservation of acetylcholine released at motor-nerve endings. Now, if the muscle is denervated, the depolarization produced by TEPP is not completely abolished. But this is not due to a direct action by TEPP, since if you now take blood from the animal, there is acetylcholine in it, enough to produce the effect recorded, and running a similar time course. In poisoning by TEPP, therefore, one's muscles are paralysed first by the acetylcholine released by the activity in one's own motor nerves, and secondly (with much deeper poisoning) by acetylcholine accumulating in the blood (from the portal system and elsewhere).

Although qualitatively one can account for the actions of inhibitors, there is often a failure to relate symptomatology quantitatively to the degree of cholinesterase inhibition. One can find abundant evidence, for instance, that the serum cholinesterases can be almost completely inhibited without producing symptoms. Again, in animals, there is no, or only a poor, correlation between intensity of symptoms and the reduction of brain or red-cell true cholinesterase (Frawley *et al.*, 1952). But it needs only a little reflection to see how hard it is to test such a correlation fairly: one needs to know the degree of inhibition of the cholinesterase at the particular site of interest, for it is known that it may vary considerably from tissue to tissue. The most detailed attempts, by Riker and Wescoe (1949) on the salivary gland, and by Koelle and colleagues (Koelle *et al.*, 1950; Kamijo and Koelle, 1952) on the ganglion and small intestine, have been more encouraging. It is disappointing that we cannot judge cholinesterase concentrations in the recesses of the brain or the muscles from assays on readily accessible serum or red cells, but it was rather optimistic to expect it.

On the whole, then, one can retain the belief that most of the actions are due to acetylcholine accumulation, and look to further work to establish the parallelism of inhibition and effect.

There is one result of giving cholinesterase inhibitors of a different kind, with some potential clinical importance. Serum cholinesterase can hydrolyse, quite rapidly, two other substances, succinylcholine, and local anaesthetics, especially procaine. After the administration of an inhibitor, it would be quite wrong to expect succinylcholine still to be transient in action, or to suppose that it will be possible to inject such large amounts of procaine into tissues (for local anaesthetic purposes) as one can normally do without producing systemic effects.

Demyelination by Anticholinesterases

It was, as described above, at first believed that chronic administration of anticholinesterases produced (except for a hypoplasia of spleen and thymus) no morphological effects other than those due to the usual accumulation of acetylcholine. This is still, generally speaking, true. But as a result of exposure to certain insecticides a number of cases of curious paralysis have been described which have led to the discovery that at least two of the newer anticholinesterases are able to produce, in animals, a demyelination of parts of the nervous system. These are DFP and Mipafox. The lesion is the same as was seen some years ago when TOCP was drunk in a ginger wine in the States;

and the analogy became still more striking when it was found that TOCP was a powerful inhibitor of pseudo-cholinesterase, particularly of the white matter of the brain. It is seen particularly clearly in hens in which lesions, chiefly of the spino-cerebellar tract but also of other fibre groups and of sciatic nerve, have been produced; demyelination of the sciatic nerves of rats and rabbits has also been shown. This lesion does not come on for something like one or two weeks; so that in the hen after DFP there is a characteristic progression from the typical poisoning in a hen due to an acetylcholine-like action, with paralysis and stiffly extended limbs, moving on later to ataxia and an incomplete motor palsy quite unlike the initial picture (Barnes and Denz, 1952).

The human cases after Mipafox followed a course rather like the hen (Bidstrup, Bonnell and Beckett, 1953). After the exposure to the insecticide the subjects complained of vomiting and diarrhoea and nausea; their pupils were tightly constricted and conjunctivae flushed; their muscles, particularly in the face and neck, were twitching and the whole musculature was weak. They were treated with atropine, 1/50th of a grain roughly every two hours which relieved the muscarinic symptoms but did not influence the muscular weakness and general hypotonia. Then about 15 days after the original exposure an ataxia was noticed. This slowly developed, producing a considerable weakness in the legs and a development of weakness in the hands and arms. Clinically there was a flaccid paralysis of the legs, the muscles were tender, there were twitchings in many muscles. Sensation was normal, tone considerably reduced. At this point there were no fibrillation potentials and the electromyographer thought the condition was compatible with a depolarization neuromuscular block. A particularly interesting point was that an attempt at physiotherapy, the usual treatment adopted for such cases, led to a very severe deterioration with increased paralysis, generalized twitchings and clonic spasms. Later there was an obvious atrophy of some of the muscles, particularly small muscles of the hand, but eventually recovery began and has progressed continuously.

A second case had a slightly different onset with wheezing and tightness in the chest and ptosis (rather than the abdominal symptoms of the first case). This progressed to severe weakness and hypotonia but was successfully treated in the acute stage with atropine, the patient being discharged about a fortnight later. About a week after discharge, however, he noticed cramp-like pains in the calves and feet when riding a bicycle and began to get rather weak in his legs. In hospital he was found to have a partial motor paralysis with tender muscles but was electromyographically fairly normal. With lapse of time some recovery took place but he remained easily tired for a considerable time.

In animals it is known that a good many other potent anticholinesterases cannot produce this lesion. Nevertheless the fact that three agents, TOCP, Mipafox and DFP, of this group can do so, means that it has to be taken into serious consideration as a possible result of any cholinesterase action. Two rather different questions arise here. Taking the experimental demyelination first, *is it due to cholinesterase inhibition?*

The argument for it is that all the agents producing it are cholinesterase inhibitors; and indeed, because TOCP is particularly an inhibitor of pseudo-cholinesterase in the central nervous system it has been suggested that the demyelination is particularly due to interference with this enzyme, supposing it to have some special relationship to the nutrition of nervous tissue. But against this supposition are three points: (*a*) that there are at least six other anticholinesterases which do not induce demyelination; (*b*) that there is no parallel between the intensity and course in time of the effect of the inhibitor on the tissue cholinesterases and the actual appearance of the demyelination; (*c*) TOCP, although it is an anticholinesterase, does not, when given in doses which produce this paralysis, elicit any of the usual cholinergic symptoms. One is left suspecting therefore that the demyelination is not due to cholinesterase inhibition but rather to the inhibition of some other enzyme by these particular compounds. Remembering that they are not entirely specific, this is not unlikely.

Secondly, in the clinical cases, *is the motor paralysis due to inhibition of cholinesterase?* It seems reasonably clear that the initial weakness could be attributed to it, since it was known that the blood cholinesterase was reduced, that there were often fasciculations and cramps, and that the condition worsened with muscular activity. But it is difficult to see how cholinesterase inhibition by itself, at the motor endplates, can account for a sequence of events whereby the initial muscular weakness virtually passes off and is then re-established again accompanied by wasting of muscles and ultimately muscular fibrillations, without any signs of cholinesterase inhibition anywhere else in the body. The whole picture suggests, in short, that for the first week or two there is a true antiesterase neuromuscular block but that this slowly moves over into a paralysis due to a partial denervation possibly produced by a destruction of nerve trunks remote from the muscle.

Whatever may be the truth of these speculations, two practical points arise: *first*, that every case of anticholinesterase poisoning should be followed for one or two months after the acute stage has been resolved; and *second* that if a paralysis does develop, it should be treated rather cautiously and without the vigorous physiotherapy so useful in other lower motor neurone lesions.

Treatment of Myasthenia Gravis

The basis of therapy of myasthenia gravis by antiesterases is fairly well agreed. Until recently neostigmine was virtually the only drug of importance, being much superior to eserine because of its greater proportionate effects on muscles. Its derivative—tensilon—has not really supplanted it, despite some attractive features, because it tends to be too transient in action. A weakness of neostigmine therapy, however, is that the patient is condemned to continual ups and downs in his muscular strength, because of the fairly short duration of action of the drug. When the irreversible inhibitors appeared, therefore, it was hoped that these might allow a smoother, more continuous control. DFP, the first one used, was reasonably effective and gave a more sustained action than neostigmine; but it did not produce as good a result as neostigmine at the peak of its action, and in addition exerted the somewhat unpleasant central effects previously mentioned. More promising has been the use of OMPA which, because of its low fat solubility, does not penetrate the central nervous system. With OMPA, in a proportion of cases at least, a smooth and effective control can sometimes be established. Whether the extra trouble in controlling the dosage of a drug with such prolonged action and consequent danger of accumulation is worth while, when successful therapy with neostigmine can be achieved fairly easily, is uncertain. But what I wish to mention here is a very instructive instance of how confusing neuromuscular paralysis may be.

This can be illustrated with a case from America (Wilson *et al.*, 1952) of a man not responding very well to neostigmine, so that it was decided to try him on OMPA. He was started on 7 mgm. OMPA twice a day orally together with a neostigmine supplement, 1 mgm. approximately 4-hourly intramuscularly. In about a week his serum cholinesterase was down to 19 per cent of normal and red-cell cholinesterase to 10 per cent of normal. The control was still not adequate and the OMPA was increased to 14 mgm. twice a day, atropine was given to control salivation and neostigmine was slowly stopped. So far all was well. Then the patient began to get weaker again and neostigmine was given promptly intramuscularly to overcome what was thought *might* be a 'myasthenic crisis'. A few hours later the patient was weaker still, and the doctor in charge, now convinced that this was a true 'myasthenic crisis', gave yet more neostigmine intramuscularly. The patient now became weaker still, unable to swallow the saliva which was pouring from his mouth faster than it could be sucked out and sweating so hard that the bed linen was wringing wet. Fortunately, a consultation now took place and the diagnosis was made of a 'cholinergic crisis'. Administration of anticholinesterases was immediately stopped; 1/50th of a grain of atropine was given intravenously; and the patient was put in an oxygen tent. The atropine was repeated every 4 hours and in 36 hours he had greatly improved. Eventually he was returned to a maintenance dose of 10 mgm. of OMPA night and morning.

There are three interesting points here. *First*, that the onset of poisoning by an anticholinesterase can occur in a myasthenic patient, and appears superficially as an intensification of the myasthenic process. *Secondly*, it was noted that there was no useful warning change in the cholinesterases values of serum and blood cell. These remained at more or less the same rather low levels both before and during the crisis. *Third*, atropine appears to have been an excellent antidote and fully justified its universal recommendation. There are indications that similar crises occur when over-enthusiastic attempts are made to antagonize the effects of curarizing compounds given during surgical anaesthesia.

Accommodation to Anticholinesterases

It must not be assumed that the body accepts the insults of anticholinesterase administration completely passively. There are two interesting bits of evidence pointing to the contrary. First, it has been found that after the administration of a number of these inhibitors, the depression of cholinesterase activity may be succeeded by a rise in cholinesterase activity of a tissue. The conditions governing this seem still somewhat obscure but it is an interesting finding and if it can be confirmed, suggests that a sort of hypertrophy of the cholinesterase system can occur in response to a burden laid upon it (Locker and Siedeck, 1952).

A better established observation with a number of inhibitors is that, if an animal can be tided over the first exposure, he may become relatively resistant to a subsequent one. For instance, Barnes has shown (1953) that if artificial respiration was given after a normally lethal dose of paraoxone, recovery takes place in approximately 25 minutes. If now the dose is repeated half an hour or an hour later, there is less muscular fasciculation and collapse and the depression of the breathing is slower and less intense. Corresponding to this is an observation made by Douglas and Paton (1954) that the depolarization of the motor endplate produced by large doses of TEPP tends to wane with successive administrations. This is not because the amount of acetylcholine accumulating in the blood changes, for one can find just as high levels after subsequent doses as after the first one. There seems to be some real accommodation by the endplate to acetylcholine of the same sort as Burns and Paton (1951) observed with decamethonium at the endplate. This accommodation seems to be restricted to the neuromuscular and respiratory effects rather than to the muscarinic actions, and so far as broncho-spasm or fall in blood pressure contribute to the lethal process, it may not be much reduced.

Antagonists to the Effect of Cholinesterase Inhibitors

Cases of poisoning by anticholinesterases may occur during the use of insecticides in agriculture, during treatment of myasthenia, during surgical anaesthesia and (perhaps) in wartime, so that the importance of treatment of such poisoning needs no emphasis. There is some debate whether death, when it occurs, is due to central respiratory depression, peripheral neuromuscular block, bronchospasm, circulatory failure, or pulmonary oedema, or to an assortment of these. But this is a somewhat academic debate, since cases vary so much that one must clearly be ready for all the important manifestations of the poisoning.

The pre-eminent remedy is atropine, principally for the muscarinic actions (cf. Grob, 1950). It has repeatedly proved itself able to relieve or abolish these and relatively large doses can be given. The usual advice is to give 1/50th of a grain intravenously if necessary, perhaps every 15 minutes if poisoning is severe, until there are clear signs of atropinization, such as dilation of the pupil. Atropine has little effect on the neuromuscular block but it is claimed that it can relieve the depressant effect of anticholinesterases on the central nervous system (Douglas and Matthews, 1952). Whether this is important clinically is not established, but it is certainly not an undesirable effect.

The treatment of neuromuscular paralysis is distinctly harder. It is known that d-tubocurarine can prevent many of the effects of inhibitors at the neuromuscular junction; but for practical purposes the obvious difficulty occurs that it itself produces neuromuscular block, so that although the inhibitor has been antagonized, the patient is little better off. Parkes and Sacra (1954) have shown that hexamethonium can increase the protection given by atropine (normally about two-fold) to as much as ten-fold, and that it confers considerable protection by itself (this may well be due to its feeble neuromuscular action which forms the basis for its original suggested antagonism to decamethonium). The protection by atropine and hexamethonium together can thus be a notable one, although it is less striking when given *therapeutically*.

A number of other compounds have been suggested as antagonists. Nikethamide, for instance, although itself useless as a protective agent, when given to rats together with atropine (on the theory that it would diminish the central depressant effects) was found to increase atropine's protective effect by eight-fold in female rats and two-fold in male rats (DiStefano *et al.*, 1951). Similarly Heymans (1950) has claimed that Diparcol and some of the other nicotinolytic drugs can confer a useful protection. Other analeptics, such as metrazol, benzedrine, picrotoxin or caffeine, are all believed to increase mortality.

The general conclusion is that atropine is outstanding as a therapeutic agent, that hexamethonium might be tried cautiously where there is confidence that neuromuscular block is an important element in the poisoning, but that the use of other agents should be deferred until more work has been done. It is, of course, a truism, but one worth repeating, that other measures in the treatment of a patient should not be forgotten: (1) Artificial respiration with oxygen to counter the effects of respiratory paralysis, bronchospasm (cf. Holmstedt, 1951), or pulmonary oedema; (2) appropriate measures for raising the blood pressure if this sinks very low and cannot be controlled by atropine (although this seems very rare); and (3) care that the patient is not getting cold; this may easily happen with profuse sweating, and perhaps some peripheral cutaneous vasodilation.

One may conclude, perhaps, by summarizing the possible ways of protection available at present against such 'nerve gases' in this way. Let us suppose that you are a sufferer from Parkinson's disease (therefore taking diparcol), a severe hypertensive (therefore on hexamethonium), a myasthenic (therefore taking neostigmine and atropine), and with cirrhosis of the liver (and therefore unable to activate Parathion or OMPA); then you may well feel proud, for if there is another war, your extraordinary resistance to these war poisons will ensure your early despatch not to hospital, but to the 1st Commando regiment.

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